MEDIUM FOR ISOELECTRIC FOCUSING

The present invention relates to a medium for isoelectric focusing, which medium is based on agarose.

An important area of use of the medium according to the invention is as anti-convection medium or stabilizing medium in analytical isoelectric focusing. At present the predominant medium for this type of isoelectric focusing is cross-linked polyacrylamid (polyacrylamide gel). It affords stable pH gradients and excellent separation of proteins but it also has some important draw-backs. The procedure for the preparation of the polyacrylamide gel is laborious and includes the use of very toxic chemicals. Another draw-back is that the polyacrylamide gel consists of so tight a net-work of polymer chains that the migration of the proteins in the electrical field is retarded and large proteins with molecular weights above 100,000 can not as a rule be focused because of the small size of the meshes.

Agarose has been proposed as an alternative to the polyacrylamide. However, agarose contains negatively charged groups, mainly sulphate ester groups and carboxyl groups (with positive counter ions such as alkaline metal ions, e.g. sodium or potassium ions, or ions of organic amines), which causes the so called electroosmosis, i.e. a flow of liquid through the gel. This causes the position of the pH gradient of move continously in the gel and eventually the gradient will degrade completely. Very often parts of the gel become dried out and collapse due to the electroosmosis.

It has been suggested that agarose gels suitable for isoelectric focusing can be prepared by purification or chemical modification using various methods or by using polymeric additives to increase the viscosity in order to reduce the electroosmotic flow, see for example Johansson, BG and Hjerten, S, Anal. Biochem. 59 (1974) 200, Weise, H-Ch and Grässlin, D, Acta Endocrinol. Suppl. 82 (1976) 75, and Grubb, A, Anal. Biochem. 55 (1973) 582 and the corresponding lecture abstract in Proceedings of the Twenty-First Colloquium on Protides of the Biological Fluids, Brügge, 1973, p. 649, (ed H Peters, Pergamin Press, 1974). The proposed purification and modification methods have, however, not resulted in complete elimination of the electroosmosis problem and the same holds also for the addition of polymers, which also reduces some of the advantages of agarose.

According to the present invention it has now been demonstrated that the net charge of the agarose matrix can be modified by the introduction of positively charged groups of a type which renders the agarose very suitable for use in isoelectric focusing.

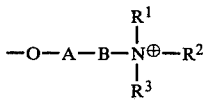
The medium according to the invention is characterized in that it consists of or contains agarose into which positively charged substituents have been introduced to the neutralisation of negatively charged groups present in the medium, said substituents containing as the only charged group a quaternary amino group and the charge of said substituents being independent of pH at least in the range 2-12.

The substituents are according to a preferred embodiment of the medium according to the invention bound to the agarose via an ether or carboxylic acid ester linkage involving the oxygen atom of a hydroxyl group of the agarose.

Preferably the substituents do not contain other nitrogen atoms than those of the quaternary amino groups.

Inorganic or organic negative ions, e.g. chloride ions, nitrate ions, sulphate ions or negative ions of organic acids, can be chosen as counter ions to the above mentioned quaternary amino groups. The negative ionic groups present in the agarose itself can also serve as counter ions.

According to a particularly preferred embodiment of the medium according to the invention the positively charged substituents introduced into the agarose matrix are those of the formula



wherein R^1, R^2, R^3 are equal or different, and each represents an alkyl group containing 1–5 carbon atoms, a hydroxyalkyl group containing 2–5 carbon atoms, an aryl or an aralkyl group containing 1–5 carbon atoms in the alkyl part, or R^2 may together with R^1 or R^3 and the quarternary nitrogen atom form a heterocyclic ring, which ring also can carry an oxygen atom in the ring separated from the nitrogen atom by two carbon atoms, A represents a single bond or a carbonyl group —CO—, and B is an alkylene chain containing 2–10 carbon atoms in the case of A representing a single bond and 1–10 carbon atoms when A represents a carbonyl group, which alkylene chain represented by B may be interrupted by one or more ether groups and may be substituted by one or several alkyl and/or hydroxyl groups, at most one heteroatom being bound to one and the same carbon atom in the chain, and wherein the oxygen atom (—O—) originates from a hydroxyl group in the agarose. (The term "heteroatom" refers to other atoms than carbon and hydrogen atoms.) The counter ions to the substituents of this general formula may be those mentioned above.

The benzene ring can be unsubstituted or substituted in cases, where R^1, R^2 and/or R^3 are representing an aryl or an aralkyl group. In the latter case the substituents will of course be of a type which fulfils the requirement for pH independent positive charges. Examples of such groups are alkyl and alkoxy groups containing 1–6 carbon atoms and hydroxyalkyl groups containing 1–6 carbon atoms.

The following groups are examples of substituents of the general formula given above: